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## **Humanised mouse models for haematopoiesis and infectious diseases**

Lysenko, Veronika ; McHugh, Donal ; Behrmann, Lena ; Rochat, Mary-Aude ; Wilk, Christian M ; Kovtonyuk, Larisa V ; Bourquin, Jean-Pierre ; Münz, Christian ; Manz, Markus G ; Speck, Roberto F ; Theocharides, Alexandre P

**Abstract:** “Humanised” mouse models have emerged over past years as powerful tools for investigating human haematopoiesis and immunity. They allowed the identification of key factors for the maintenance and function of normal and leukaemic human haematopoietic stem cells. These findings have been widely used to dissect the pathogenesis of multiple myeloid and lymphoid neoplasms, such as acute myeloid leukaemia and acute lymphoblastic leukaemia. Furthermore, these models can serve as a stepping-stone to clinical trials by testing novel drugs that target leukaemic stem cells. The investigation of human immunity in vivo is also of great interest in both the context of understanding the innate and adaptive immune system and responses to viral infections with exclusive human tropism, such as Epstein-Barr virus and human immunodeficiency virus. This review focuses on recent advances in the study of human haematopoiesis and immunity in humanised mouse models, underlining their relevance and limitations. **Key words:** humanised mouse models, xenograft, leukaemia, HIV, EBV, PDX

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## Humanised mouse models for haematopoiesis and infectious diseases

Lysenko Veronika<sup>a</sup>, McHugh Donal<sup>b</sup>, Behrmann Lena<sup>c</sup>, Rochat Mary-Aude<sup>d</sup>, Wilk Christian M.<sup>a</sup>, Kovtonyuk Larisa<sup>a</sup>, Bourquin Jean-Pierre<sup>c</sup>, Münz Christian<sup>b</sup>, Manz Markus G.<sup>a</sup>, Speck Roberto<sup>d</sup>, Theocharides Alexandre P.A.<sup>a</sup>

<sup>a</sup> Haematology, University Hospital Zurich and University of Zurich, Switzerland

<sup>b</sup> Viral Immunobiology, Institute of Experimental Immunology, University of Zurich, Switzerland

<sup>c</sup> Paediatric Oncology, Children's Research Centre, University Children's Hospital, University of Zurich, Switzerland

<sup>d</sup> Division of Infectious Diseases and Hospital Epidemiology, University Hospital of Zurich, University of Zurich, Switzerland

### Summary

"Humanised" mouse models have emerged over past years as powerful tools for investigating human haematopoiesis and immunity. They allowed the identification of key factors for the maintenance and function of normal and leukaemic human haematopoietic stem cells. These findings have been widely used to dissect the pathogenesis of multiple myeloid and lymphoid neoplasms, such as acute myeloid leukaemia and acute lymphoblastic leukaemia. Furthermore, these models can serve as a stepping-stone to clinical trials by testing novel drugs that target leukaemic stem cells. The investigation of human immunity *in vivo* is also of great interest in both the context of understanding the innate and adaptive immune system and responses to viral infections with exclusive human tropism, such as Epstein-Barr virus and human immunodeficiency virus. This review focuses on recent advances in the study of human haematopoiesis and immunity in humanised mouse models, underlining their relevance and limitations.

**Key words:** humanised mouse models, xenograft, leukaemia, HIV, EBV, PDX

### Introduction

The need to understand normal or malignant human cell development and disease heterogeneity, and to investigate novel therapeutic strategies in order to accelerate their transition into clinical trials, strongly supported the development of "humanised" mouse models [1, 2]. Another important driving force for the development of these models is the ethical concerns raised by clinical trials in humans. Three major advancements over past decades have significantly shaped the field (fig. 1). The first was the discovery of a spontaneous autosomal recessive mutation in the *Prkdc* gene, termed *Prkdc<sup>scid</sup>* (*Prkdc*: protein kinase, DNA activated, catalytic polypeptide; *scid*: severe combined immunodeficiency) in C.B-17 mice (called SCID mice). This permitted low engraftment of human fetal tissues, peripheral blood mononuclear cells and haematopoietic stem cells (HSCs) owing to a T and B cell deficiency

[3–5]. Low human haematopoietic engraftment in C.B-17 mice is due to a tendency to develop functional mouse T and B cell immunity (termed "leakiness") and normal natural killer (NK) cell activity [6]. Furthermore, mutations in the locus of one of the recombination-activating genes (RAG1 or RAG2) resulted in *Rag1<sup>tm1Mom</sup>* or *Rag2<sup>tm1Cgn</sup>* mice with a T- and B-cell deficiency similar to that seen in C.B-17 mice, but in addition rendered them resistant to radiation and leakiness [7].

The second advancement was the development of NOD.CB17-*Prkdc<sup>scid</sup>*/J mice, generated by backcrossing mice with the *Prkdc<sup>scid</sup>* mutation to mice on the NOD (non-obese diabetic) background [8]. In comparison to the C.B-17 mice, these mice showed improved engraftment of HSCs as a result of lower NK cell activity and defects in their innate immunity, although they had short life-spans due to spontaneous development of thymic lymphomas [8].

The introduction of a targeted mutation in the locus of the interleukin-2 receptor (IL-2R)  $\gamma$ -chain, which leads to NK cell depletion, was the third key achievement that led to a substantially higher engraftment of functional human HSCs [9–12]. Today, the NOD.Cg-*Prkdc<sup>scid</sup>* *Il2rg<sup>tm1Wjl</sup>*/SzJ (NSG) model that resulted represents the most commonly used host strain for xenograft experiments. Although the introduction of NSG mice has significantly advanced the field, NSG mice fail to support maturation of human B cells into memory and plasma cells. In addition, T cell development lacks thymic support and the development of innate immunity (i.e., NK cell and macrophage development) is limited [13].

A crucial immune determinant of human haematopoietic cell survival identified in xenograft models is macrophage tolerance [14, 15]. Binding of human CD47 on human haematopoietic cells to mouse signal regulatory protein- $\alpha$  (SIRP $\alpha$ ) on resident host macrophages generates a "don't eat me" signal that leads to inhibition of phagocytosis, protects the transplanted human cells and consecutively contributes to the human cell survival *in vivo* [15, 16]. The interaction between human CD47 and mouse SIRP $\alpha$  is present in mice on a NOD background since the mouse

### Correspondence:

Alexandre Theocharides, MD and Markus G. Manz, MD, Haematology, University Hospital Zurich and University of Zurich, CH-8091 Zurich, Switzerland, alexandre.theocharides[at]usz.ch; markus.manz[at]usz.ch

SIRP $\alpha$  IgV domain is very similar to the human homologue.

In addition, the recognition of the importance of cytokines and growth factors secreted by bone marrow stromal cells in supporting HSC development led to the development of “next-generation” humanised mouse strains, such as MISTRG mice [17]. These mice were genetically engineered to express human macrophage colony stimulating factor (M-CSF), interleukin-3 (IL-3), SIRP $\alpha$ , thrombopoietin and granulocyte-macrophage-CSF (GM-CSF) (abbreviated MISTRG) to allow efficient human haematopoietic development. In contrast to previous models, these support the development of functional human macrophages and NK cells [17]. Moreover, these mice favour myelomonocytic differentiation and the engraftment of mobilised peripheral blood mononuclear cells, which is limited in the NOD.CB17-*Prkdc*<sup>scid</sup>/J and NSG mouse models [18]. Recently, an improved “next-generation” humanised mouse strain of MISTRG that harbours an additional knock-in of human interleukin-6 (IL-6) was developed. These mice support efficient engraftment of human plasma cell neoplasms, which particularly rely on the presence of IL-6 [19, 20]. Improved models that support multilineage engraftment of human HSCs while minimising the mouse HSC compartment by ablating mouse cells were also developed. These models were generated by the introduction of mutations in the Kit receptor, which is vital for HSC maintenance and function, in immunodeficient mouse strains on the BALB/c (BRg *Kit*<sup>Wv/Wv</sup>) or NOD (NSG *Kit*<sup>Wv/Wv</sup>) background [21].

Although the humanised mice field has advanced extensively, there are still obstacles that need to be overcome in order to generate a model that fully recapitulates the complexity of the human haematopoietic system. This review

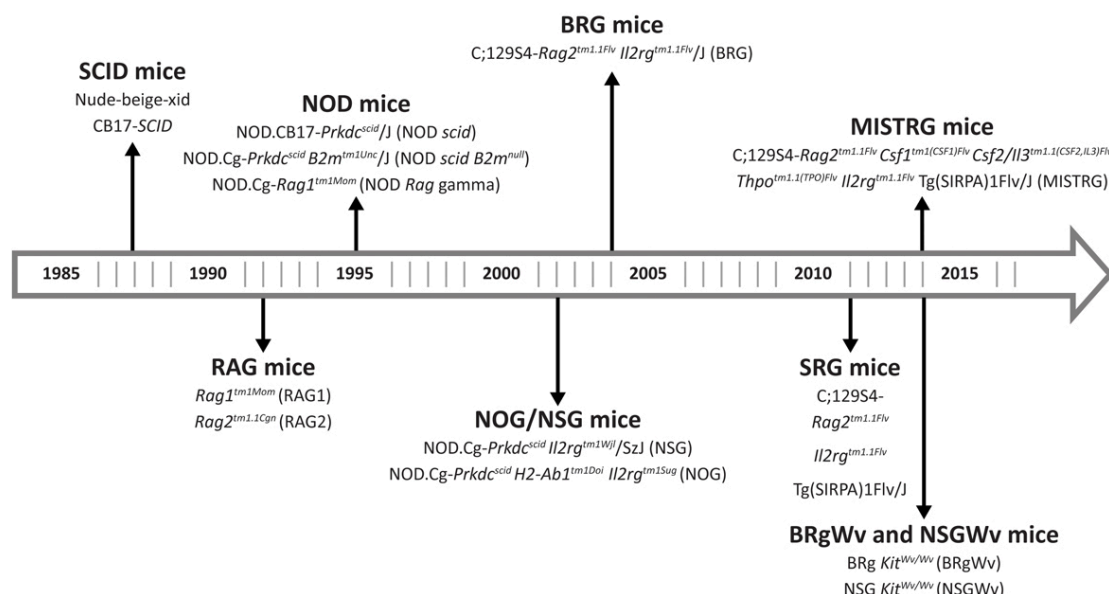
focuses on current advances in the development of humanised mouse models for human haematopoiesis and immunity, highlighting their relevance and limitations.

## Haematopoiesis

### Normal human haematopoiesis

The haematopoietic system is a hierarchically organised cellular system, in which HSCs reside at the apex and are characterised by their ability to self-renew and to differentiate into mature blood cells of the myeloid and the lymphoid lineage (fig. 2 and table 1). By definition, human HSCs are capable of repopulating immune-compromised mice and are enriched in the lineage-marker negative (Lin<sup>−</sup>), CD34<sup>+</sup>, CD38<sup>−</sup>, CD90<sup>+</sup> (Thy1<sup>+</sup>) and CD45RA<sup>−</sup> population in the human fetal liver / fetal bone marrow, umbilical cord blood and adult bone marrow [24–30]. Later, CD49f integrin was identified as a marker that further enriches for human HSCs in umbilical cord blood samples. In combination with the high efflux of the mitochondrial dye rhodamin 123, 14 to 28% (i.e., 1 in 3.5–7 cells) of functional umbilical cord blood human HSCs that express the surface phenotype mentioned above are capable of engrafting NSG mice at the single cell level [22]. HSCs give rise to multipotent progenitors (MPPs), which in turn give rise to oligopotent multilymphoid progenitors (MLPs) and common myeloid progenitors (CMPs), which further differentiate into unipotent progenitors (fig. 2). For example, CMPs give rise to lineage-committed megakaryocyte/erythrocyte progenitors (MEPs) and granulocyte/monocyte progenitors (GMPs). MPPs are distinguished from HSCs by the lack of CD49f expression (table 1) [22]. Recently, the surface marker profile of CMPs, MEPs and MPPs was re-defined by the addition of CD71 and the thrombopoi-

**Figure 1: Historical development of humanised mouse strains.** The first major advancement was the discovery of a mutation in the *Prkdc* gene that led to development of SCID mice. The second was the development of NOD.CB17-*Prkdc*<sup>scid</sup>/J (NOD *scid*) mice, generated by back-crossing mice with the *Prkdc*<sup>scid</sup> mutation to mice on the NOD (non-obese diabetic) background. The introduction of a targeted mutation in the locus of the interleukin-2 receptor (IL-2R)  $\gamma$ -chain led to the development of the NOD.Cg-*Prkdc*<sup>scid</sup> *Il2rg*<sup>tm1Wjl</sup>/SzJ (NSG) mice, which represents the most widely used host strain so far. Further discoveries, took into account the importance of cytokines and growth factors in supporting HSC development, which led to the development of “next-generation” humanised mouse strains, such as MISTRG mice.



etin receptor, which allowed better purification from the rest of the populations in the haematopoietic tree [31]. Furthermore, it was shown that cells with megakaryocyte/erythroid potential branch out directly from HSCs, rather than from MEPs, a concept that resembles the mouse haematopoietic tree [32].

The regulation of HSC self-renewal and differentiation by cell-intrinsic and cell-extrinsic factors has been extensively investigated in the mouse system using knockout models, whereas their human analogues have not been explored because of the lack of suitable humanised model. Nonetheless, some key human HSCs factors have been cross-confirmed and/or newly identified. HSCs are a quiescent cell population that mostly reside in the G0 phase of the cell cycle [33]. Recently, it was demonstrated that cyclin-dependent kinase C (CDK6) levels regulate the kinetics of quiescence exit of human HSC subsets in xenograft models [34]. Further intrinsic factors that have been associated with the human HSC state include the transcription factors *SOX8*, *SOX18*, *HoxB4*, *HES1* and *BM1*. However, their function-

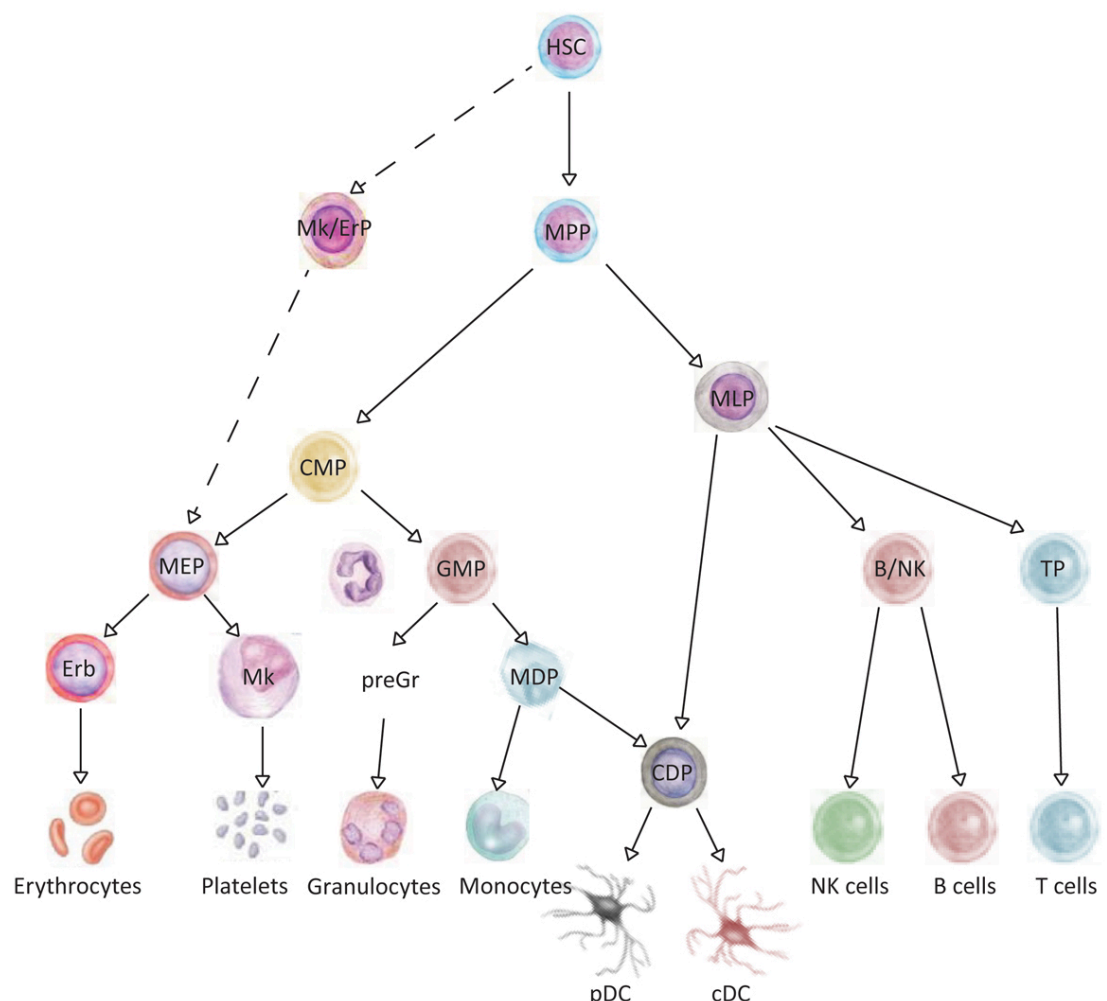
al role remains to be validated in xenograft studies. Extrinsic factors that support human HSC function and engraftment in xenograft models include the macrophage receptor *SIRP $\alpha$*  (see introduction) and thrombopoietin. Both human factors have been genetically introduced into immune-compromised mice in order to facilitate normal and malignant human HSC engraftment [35, 36]. Overall, there are still many gaps in the current knowledge of normal human HSC maintenance and function, which with the development of new humanized mouse models can be further elucidated.

## Malignant human haematopoiesis

### Myeloid neoplasms

Patient-derived xenograft (PDX) models have been extensively used to dissect the pathogenesis of acute myeloid leukaemia (AML) at the clonal level and for preclinical testing of novel therapeutic regimens. One major contribution of PDX models to the AML field is the identi-

**Figure 2: Hierarchy within human haematopoietic system.** The tree-like organisation of the haematopoietic system is composed of haematopoietic stem cells (HSCs) with long-term self-renewal capacity that reside at the apex of the hierarchy and through multipotent and lineage-committed progenitors generate the differentiated mature cells with no or limited self-renewal capacity. B/NK = B and NK cell progenitor; cDC = conventional dendritic cells; CDP = common dendritic cell progenitor; CMP = common myeloid progenitor; Erb = erythroblast; GMP = granulocyte monocyte progenitor; HSC = haematopoietic stem cells; MDP = monocyte and dendritic cell progenitor; MEP = megakaryocyte-erythrocyte progenitor; Mk = megakaryocytes; Mk/ErP = megakaryocyte/erythrocyte progenitor; MLP = multi-lymphoid progenitor; MPP = multi-potent progenitor; pDC = plasmacytoid dendritic cells; PreGr = pre granulocytes; TP = T cell progenitor



fication of preleukaemic (pre-LSCs) and leukaemic stem cells (LSCs) (fig. 3). By definition, LSCs can repopulate immune-compromised mice and propagate disease when transplanted from the primary into secondary recipients. It is well accepted that LSCs initiate disease and their persistence after therapy causes relapse in patients [38, 39]. Thus, the identification and therapeutic targeting of LSCs is of high clinical relevance.

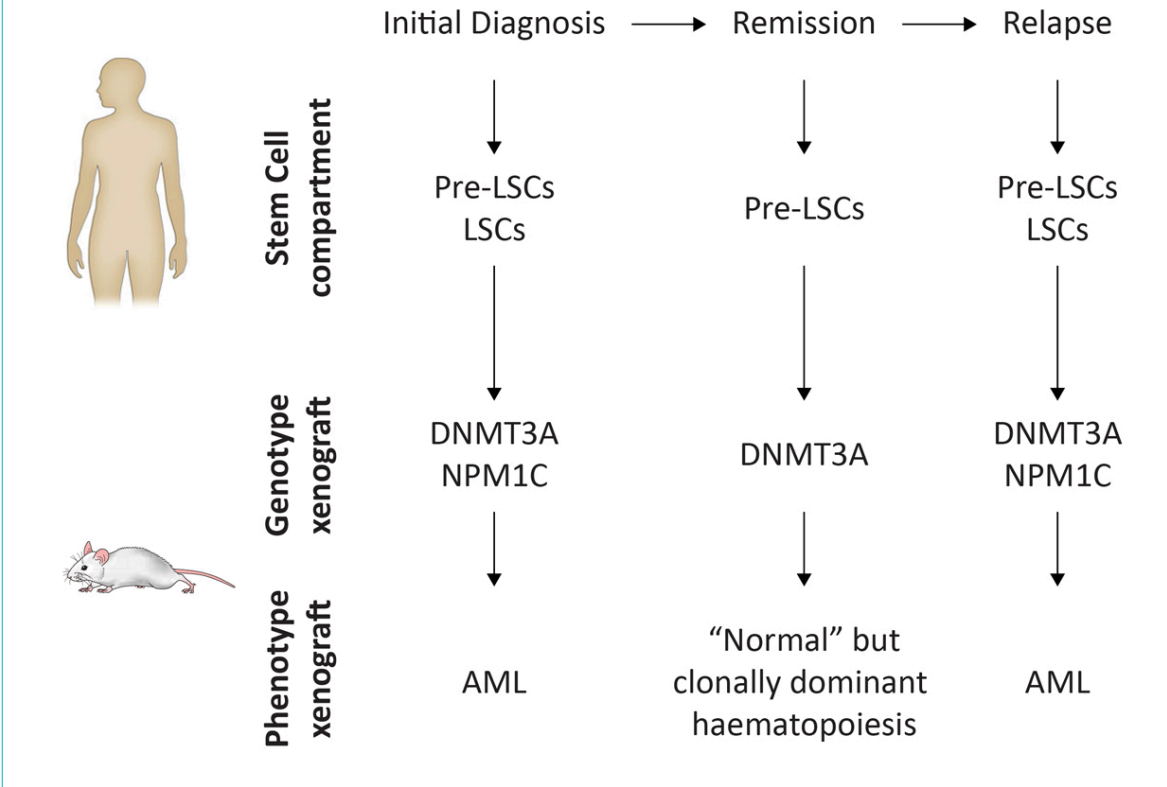
More recently, AML PDX models led to the identification of pre-LSCs. Pre-LSCs are HSCs that harbour recurrent somatic mutations and, with the acquisition of additional genetic hits, can lead to AML initiation and relapse [37, 40]. Mutations present in pre-LSCs are mostly found in genes involved in chromatin remodelling, whereas mutations that confer growth advantage to the AML clone occur later in AML development [41]. In contrast to LSCs,

Table 1: Human haematopoietic stem and progenitor cell phenotypes.

Haematopoietic cell population	Cell surface marker combination
HSPC	Lin <sup>-</sup> CD34 <sup>+</sup> CD38 <sup>-</sup>
HSC	Lin <sup>-</sup> CD34 <sup>+</sup> CD38 <sup>-</sup> CD45RA <sup>-</sup> CD90 <sup>+</sup> Rho <sup>-/low</sup> CD49f <sup>+</sup>
MPP	Lin <sup>-</sup> CD34 <sup>+</sup> CD38 <sup>-</sup> CD45RA <sup>-</sup> CD90 <sup>-</sup> CD49f <sup>-</sup>
*MPP F1	Lin <sup>-</sup> CD34 <sup>+</sup> CD38 <sup>-</sup> CD45RA <sup>-</sup> CD90 <sup>-</sup> CD49f <sup>-</sup> CD71 <sup>-</sup> BAH1 <sup>-</sup>
*MPP F2/3	Lin <sup>-</sup> CD34 <sup>+</sup> CD38 <sup>-</sup> CD45RA <sup>-</sup> CD90 <sup>-</sup> CD49f <sup>-</sup> CD71 <sup>+</sup> BAH1 <sup>-/+</sup>
MLP	Lin <sup>-</sup> CD34 <sup>+</sup> CD38 <sup>-</sup> CD45RA <sup>+</sup> CD90 <sup>-/low</sup>
CMP	CD34 <sup>+</sup> CD38 <sup>+</sup> CD45RA <sup>-</sup> CD10 <sup>-</sup> Flt3 <sup>+</sup>
*CMP F1	CD34 <sup>+</sup> CD38 <sup>+</sup> CD45RA <sup>-</sup> CD10 <sup>-</sup> Flt3 <sup>+</sup> CD71 <sup>-</sup> BAH1 <sup>-</sup>
*CMP F2/3	CD34 <sup>+</sup> CD38 <sup>+</sup> CD45RA <sup>-</sup> CD10 <sup>-</sup> Flt3 <sup>+</sup> CD71 <sup>+</sup> BAH1 <sup>-/+</sup>
MEP	CD34 <sup>+</sup> CD38 <sup>+</sup> CD45RA <sup>-</sup> CD10 <sup>-</sup> Flt3 <sup>-</sup>
*MEP F1	CD34 <sup>+</sup> CD38 <sup>+</sup> CD45RA <sup>-</sup> CD10 <sup>-</sup> Flt3 <sup>-</sup> CD71 <sup>-</sup> BAH1 <sup>-</sup>
*MEP F2/3	CD34 <sup>+</sup> CD38 <sup>+</sup> CD45RA <sup>-</sup> CD10 <sup>-</sup> Flt3 <sup>-</sup> CD71 <sup>+</sup> BAH1 <sup>-/+</sup>
GMP	CD34 <sup>+</sup> CD38 <sup>+</sup> CD45RA <sup>+</sup> CD10 <sup>-</sup> Flt3 <sup>+</sup>
B/NK	CD34 <sup>+</sup> CD38 <sup>+</sup> CD45RA <sup>+</sup> CD10 <sup>+</sup>

B/NK = B and NK cell progenitor; CMP = common myeloid progenitor; GMP = granulocyte monocyte progenitor; HSC = hematopoietic stem cells; HSPC = hematopoietic stem and progenitor cells; MEP = megakaryocyte-erythrocyte progenitor; MPP = multi-potent progenitor; MLP = multi-lymphoid progenitor; CMPs are found in cells that are CD34<sup>+</sup>CD38<sup>+</sup>CD10<sup>-</sup>Flt3<sup>+</sup>CD45RA<sup>-</sup>, MLPs are CD34<sup>+</sup>CD38<sup>-</sup>Thy1<sup>-</sup>CD45RA<sup>+</sup>, whereas MEPs and GMPs are CD34<sup>+</sup>CD38<sup>+</sup>CD10<sup>-</sup>Flt3<sup>-</sup>CD45RA<sup>-</sup> and CD34<sup>+</sup>CD38<sup>+</sup>CD10<sup>-</sup>Flt3<sup>+</sup>CD45RA<sup>+</sup> respectively [22, 23].

Figure 3: Concept of acute myeloid leukaemia (AML) xenograft models as an example for myeloid neoplasms. PDX models reflect phenotypic as well as genetic features of myeloid neoplasms. In AML, the HSC compartment consists of preleukaemic stem cells (pre-LSCs) and leukaemic stem cells (LSCs) that harbour somatic mutations. Transplantation of AML cells from the initial diagnosis into PDX models leads to AML development *in vivo*. Genetically aberrant pre-LSCs can persist in patient remission, but lead to multilineage (normal) repopulation in PDX models. Frequently, the persistence of pre-LSCs that acquire additional somatic mutations is at the origin of disease relapse, which can be reproduced in PDX models (adapted from [37]).





pre-LSCs lead to multilineage repopulation in PDX models, which makes it an excellent model to study the progression of preleukaemic lesions to overt AML.

Although PDX models significantly contributed to the AML field by enabling LSCs to be identified and characterised, the knowledge extracted from these models also answered more clinically related questions. In particular, potentially critical therapeutic factors for the survival of LSCs have been identified [42]. Among these, the immunoglobulin superfamily member CD47, which is highly expressed on LSCs, has been shown to protect LSCs from innate immune surveillance [43, 44]. Targeting CD47 in the AML PDX models demonstrated significant efficacy and constituted the basis for currently on-going clinical trials in AML, other haematopoietic neoplasms and solid tumours [45]. Furthermore, the gene expression profile of AML stem and progenitor fractions was correlated with AML development in the PDX model and led to the identification of an AML gene expression signature that correlates with clinical outcome and may be used for risk stratification in future clinical trials [46].

The relevance of AML PDX models has been questioned in the past since immune-compromised hosts such as NOD.CB17-*Prkdc<sup>scid</sup>/J* and NSG mice support engraftment of only a fraction of all AML patient samples. In particular, AML with a favourable risk profile was shown to poorly engraft conventional models. In an attempt to extend the spectrum of transplantable AML, a defined subgroup of AML characterised by the presence of an inversion of chromosome 16, excellent response to conventional chemotherapy, but poor engraftment in NSG mice, was investigated in MISTRG mice (see introduction) [47]. In contrast to NSG mice, MISTRG mice support robust engraftment of this AML subtype. The same study identified M-CSF as a critical factor for AML engraftment, a finding that could potentially be exploited therapeutically. In another study, favourable risk AML and acute promyelocytic leukaemia were successfully transplanted into engineered human bone fragments, so called ossicles, implanted into immune-deficient mice [48].

Only a few studies have successfully demonstrated engraftment of less aggressive diseases such as myelodysplastic syndromes and myeloproliferative neoplasms in immune-compromised mice. Engraftment of certain human myelodysplastic syndrome subtypes was improved by transplantation into newborn animals and by direct intrafemoral transplantation into adults [49, 50]. Other approaches include the co-transplantation of myelodysplastic syndrome samples along with healthy mesenchymal stromal cells and transplantation into ossicles (see above) [48, 51]. Similar approaches have also been undertaken for the development of myeloproliferative neoplasm PDX models. In conclusion, PDX models for myeloid neoplasms have significantly contributed to understanding the pathogenesis of AML in recent decades and today have become a standard in preclinical testing of novel therapeutic regimens. Future studies will show whether the knowledge gained from these models can be directly translated into clinical practice and help elucidate the pathogenesis of less aggressive diseases.

### Lymphoid neoplasms

Lymphoid neoplasms include acute and chronic leukaemia as well as a wide range of lymphomas that are characterised by variable stages of B and T cell maturation [52]. Acute B-precursor lymphoblastic leukaemia is a malignant disease most commonly found in children; in adulthood chronic lymphoblastic leukaemia (CLL) is more common [53]. The first leukaemia xenograft model was established more than two decades ago with AML patient samples, and provided the first functional insights into the haematopoietic hierarchy of this disease [38]. Systematic studies for acute lymphoblastic leukaemia (ALL) later set the stage for comprehensive preclinical testing [54, 55].

The development of NSG mice (see introduction) substantially improved the engraftment capacities of human lymphoma, B and T cell leukaemia [56–58]. In contrast to AML, emerging evidence indicates that most ALL cells can propagate the disease in this xenotransplantation model [59–61]. Between 50 and 100 cells are sufficient to reconstitute the leukaemic phenotype observed in the diagnostic sample [60–63]. In fact, leukaemia can be reconstituted from bone marrow samples with minimal residual disease [64] (fig. 4).

These models also contributed to the understanding of leukaemic stem cell plasticity found in certain ALL subtypes, in particular mixed lineage leukaemia (MLL)-rearranged leukaemia [65]. They further clarified the role of the MLL-rearranged ALL lineage switch to escape immunotherapeutic approaches that target CD19 on ALL cells [66]. Residual subclones that retained CD19 expression and displayed a myeloid phenotype in patients could reconstitute both a myeloid and lymphoid leukaemia in xenografts. Thus, this model can be used to address essential functional questions with leukaemia cells that are directly derived from patients. In fact, several publications have shown that leukaemia xenografts can recapitulate the clonal complexity of ALL with reasonable fidelity [60, 63, 64, 67, 68].

Recent studies indicate that this approach is also effective for adult leukaemia samples, although conditioning using total body irradiation of NSG may improve the engraftment capacities [69]. Lymphomas represent one of the most complex and heterogeneous set of malignancies and although a high diversity of PDX models are available, they fall short in truly representing patient heterogeneity [70, 71]. Additionally, the injection of lymphoma cells into specific tissues might introduce potential bias. However, tumour injection models are useful for studying the impact of different mutations and drugs on tumour aggressiveness [72–74]. Furthermore, repositories of AML, ALL and lymphoma PDXs will be available that should facilitate translational research forward [42].

The use of xenograft ALL models for preclinical testing of predictive biomarkers and drug efficacies offers new possibilities for translational research and is a big step towards clinical trials [64, 68, 75–77]. Finally, given the high degree of phenotypic and genotypic conservation between patient samples and their corresponding xenografts, this model will enable us to study the interaction of leukaemic cells within their complex niche in the bone marrow, which may provide improved and more precise rationales for therapeutic targeting of resistant leukaemia clones in their microenvironment [61, 78] (fig. 4). Xenograft models for

chronic lymphoid malignancies are also being developed using “next-generation” humanised mouse strains and will be extremely useful for preclinical testing of novel therapies [79].

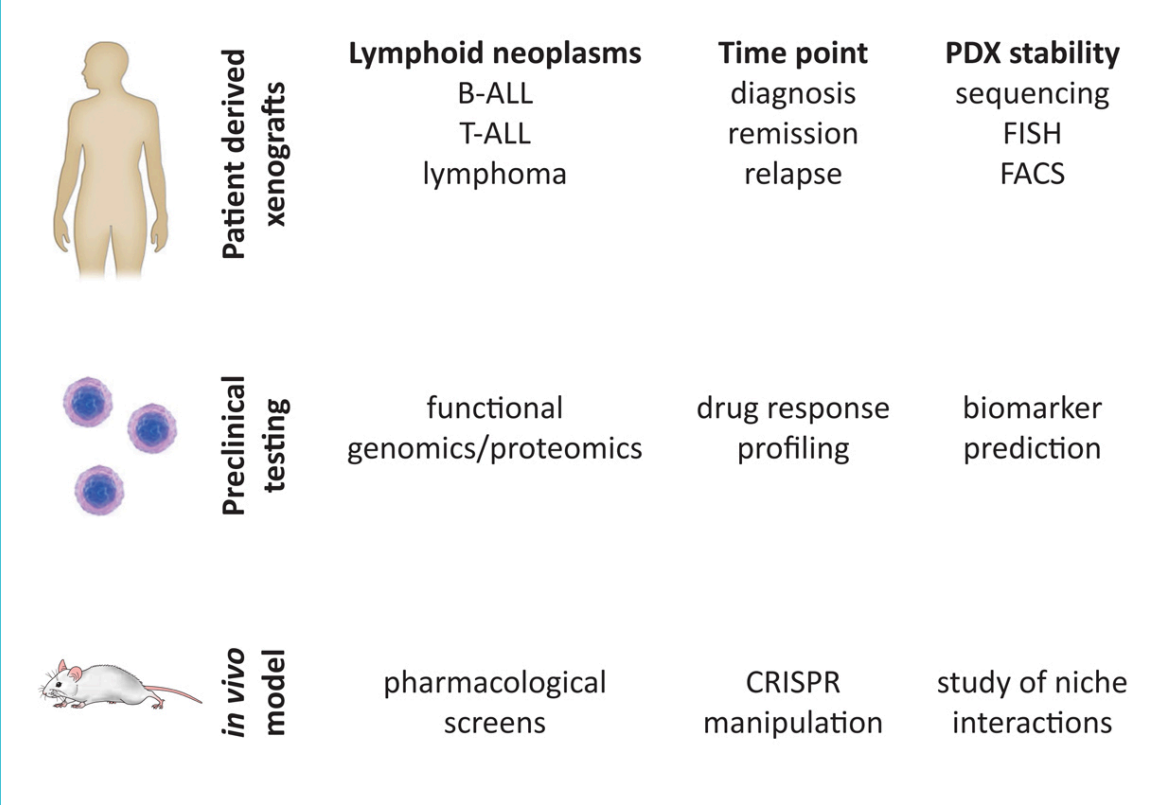
Immune system and infectious diseases

Human innate and adaptive immune responses in humanised mice

The human leucocytes of mice with human immune system components reconstituted from transferred human haematopoietic progenitor cells (HIS mice) show similarities to cord blood immune cells. Most B cells have a transitional or naïve phenotype, NK cells have a low frequency of killer immunoglobulin-like receptor (KIR) expression and still contain a CD56 negative subpopulation, and most T cells are naïve [80–83]. In addition, lymph nodes and mucosal secondary lymphoid tissues are poorly developed as a result of blocked lymphoid tissue inducer (LTi) cell differentiation in the absence of the cytokine receptor common gamma chain ( $\gamma c$ ) in most mouse strains that support efficient human immune compartment reconstitution [84]. Accordingly, cell-mediated immune responses can be mounted, while humoral immune responses are compromised, in HIS mice. Dendritic cells (DCs), the antigen-presenting cells that coordinate both cell-mediated and humoral immune responses, are reconstituted at frequencies and with a subset representation similar to that of human

secondary lymphoid tissues [85–87]. Furthermore, they react to adjuvant exposure in the form of defined toll-like receptor (TLR) ligands similar to human peripheral blood DCs, whereas mouse DCs within HIS mice react with a different hierarchy that is consistent with the dissimilar TLR distribution between mouse and man [85]. Antigen that is targeted to these human DCs with suitable adjuvants can elicit CD4<sup>+</sup> T cell responses [85, 88]. These are HLA restricted and can recognise a diverse set of antigen-derived epitopes. In addition, primed CD4<sup>+</sup> and CD8<sup>+</sup> T cell clones can target EBV-transformed B cells and, in HLA transgenic HIS mice, specificities develop which are also found in human EBV carriers [80, 89–91]. These T cell responses restrict viral loads not only during EBV infection, but also after human immunodeficiency virus (HIV) and hepatic adenovirus infection of HIS mice [80, 89, 92–94]. Therefore, protective human T cell responses can be reproduced in the humanised mouse model. In addition to priming T cells, DCs are also capable to activate innate lymphocytes. Indeed, human DC-activating adjuvants render NK cells of HIS mice more cytotoxic [81]. The early differentiated NK cell compartment of HIS mice can restrict lytic EBV infection [95] and recognises tumour cells with their distinct set of human NK cell-specific receptors [81, 96]. Furthermore, reconstituting two human immune systems in parallel from two donors that are mismatched in the HLA ligands of inhibitory NK cell receptors changes the education of these innate cytotoxic lym-

**Figure 4: Generation of PDX models for preclinical studies.** Patient-derived xenograft models of B-acute lymphocytic leukaemia (ALL), T-ALL and lymphoma can be generated from different disease time points like diagnosis, remission or relapse. PDX stability and complexity are highly conserved and can be proven by targeted sequencing, FISH (fluorescence *in situ* hybridisation) or FACS (fluorescence activated cell sort) analysis. PDX models can be used for a broad range of translation studies. Use of a co-culture system with mesenchymal stem cells enables *in vitro* approaches for drug efficacy testing or biomarker identification. The high conservation of patient samples and xenografts enables the development of *in vivo* models for pharmacological screens, cell manipulations with CRISPR or the study of niche interactions in the microenvironment of the cancer cells.

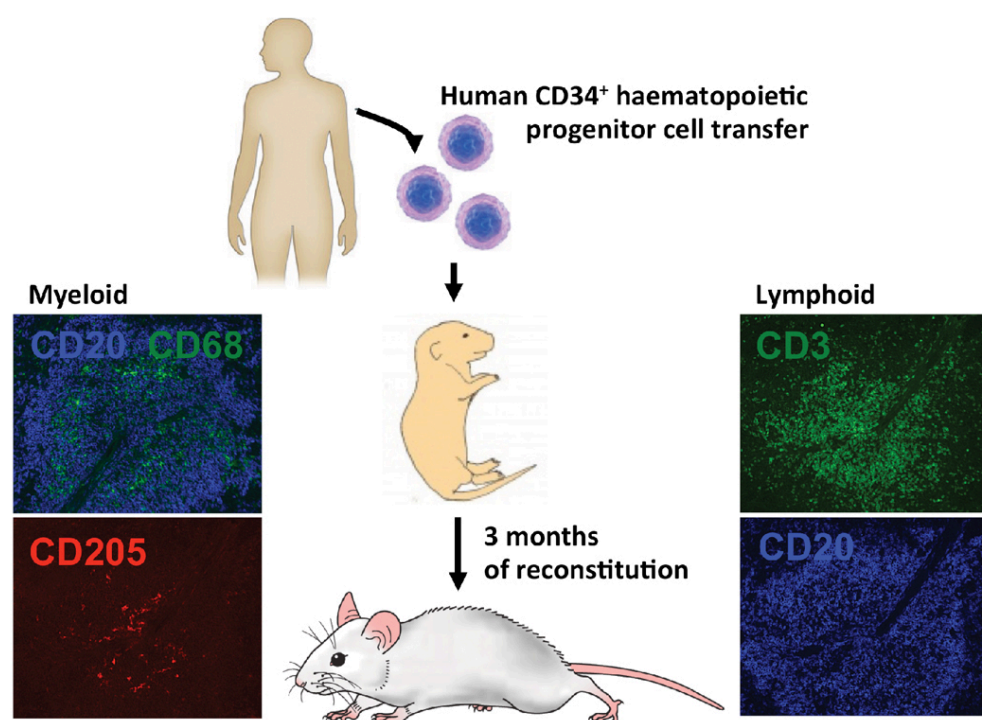


phocytes and allows the resulting NK cell population to more efficiently control EBV infection in the mixed B cell compartment [83]. This improved innate immune control most likely results from efficient targeting of EBV-infected B cells of one donor by the less inhibited NK cell compartment of the other donor. Thus, NK cell responses that are similar to those in small children can be modelled in HIS mice and manipulated for improved EBV-specific immune control.

In contrast to functional DC, T and NK cell responses, humoral immune responses are difficult to achieve. Usually only a minority of HIS mice develop specific isotype-switched antibody responses after viral infection [97]. Furthermore, the steady-state levels of human IgG in HIS mice are with below one microgram per millilitre more than a thousand-fold lower than in human serum [9, 82]. Accordingly, germinal centres are rarely observed, but even so the B and T cell zone is segregated in secondary lymphoid tissues of HIS mice (fig. 5) [85]. However, most B cells are of transitional and naïve phenotype, and follicular helper T cells that assist B cells in the germinal centre reaction are absent [82]. Interestingly, providing additional DCs either by adoptive transfer or possibly also by expansion of the reconstituted human DC populations might at least partially overcome these deficiencies and lead to B cell plasmablast differentiation, follicular helper T cell priming and antigen specific IgG responses [82]. Therefore, humoral immune responses are largely absent in HIS mice, but further manipulation of this model system might allow them to develop.

In addition to the compromised B cell compartment, it remains unclear if there is sufficient human myeloid reconstitution in HIS mice. Although DCs are found at considerable frequencies, it has been argued that macrophage populations are poorly developed [98]. Along these lines, human alveolar macrophages were able to protect HIS mice from lung proteinosis only when they developed in a human IL-3 and GM-CSF transgenic mouse background [98]. However, some lung infections could be studied in the absence of this enhanced human myeloid cell reconstitution in HIS mice. Influenza A virus infection caused the induction of virus-specific T cell responses and *Mycobacterium tuberculosis* infection led to granuloma formation [99, 100]. Therefore, whether enhanced myeloid cell reconstitution is beneficial or not might depend on the particular application of HIS mice. With respect to immune responses, it has to be kept in mind that the immunoregulatory function of some myeloid cells might be detrimental for the induction of immune responses. It has been shown that stem cell factor, IL-3 and GM-CSF transgenic mice allow elevated myeloid cell reconstitution, although at the same time expanded FoxP3 positive regulatory T cells could dampen the induction of immune responses [101]. Thus, HIS mice reconstitute a wide variety of human immune system compartments. Some of these already demonstrate functionality in the currently available models, whereas others, especially B cells, might benefit from additional manipulations of the model system in order to obtain the ability to execute their full range of immune functions. The resulting novel HIS mice may allow for a

**Figure 5: Human immune compartments of HIS mice.** HIS mice reconstitute most human immune compartments within 3 months after neonatal intrahepatic human CD34<sup>+</sup> haematopoietic progenitor cell transfer. In the spleen of NSG mice, macrophages (CD68), dendritic cells (CD205), T cells (CD3) and B cells (CD20) can be found in the white pulp.





more comprehensive investigation of human immune responses.

#### Espstein-Barr virus research with humanised mice

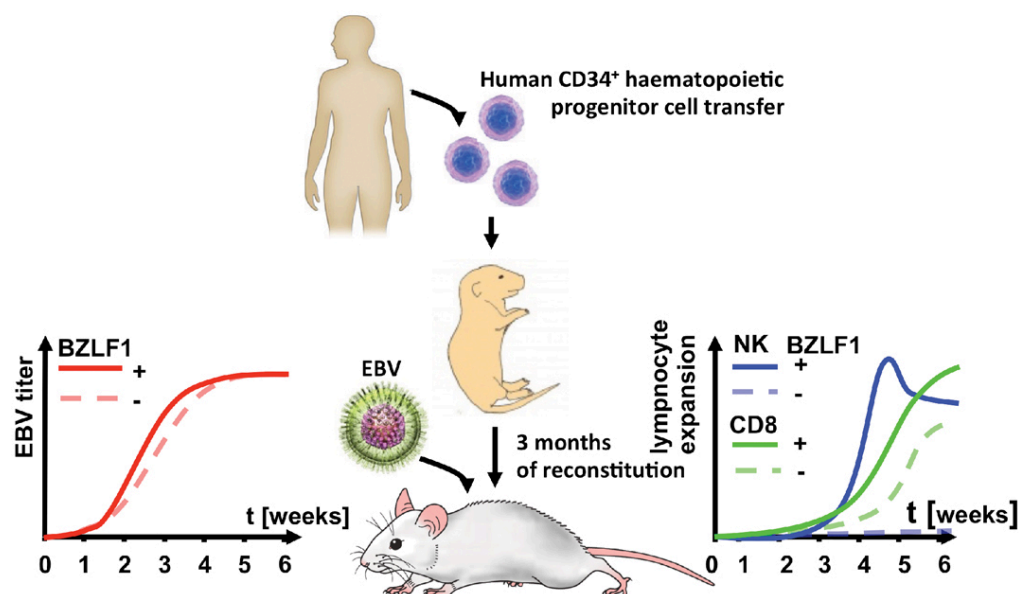
The ubiquitous gamma-herpes virus, Epstein-Barr virus (EBV), is the cause of infectious mononucleosis, and is associated with lymphomas predominantly of B cell origin and certain epithelial cell malignancies. Owing to its exclusive tropism for humans, studying this virus *in vivo* is difficult and was until recently restricted to related viruses in old-world monkeys [102]. However, EBV was not only the first human oncogenic virus to be discovered, fifty years ago [103], but it was also the first pathogenic agent to be studied in HIS mice [9]. EBV infection of B cells in HIS mice can result in asymptomatic infection or lymphoproliferative disease, depending on the infectious dose [80, 104]. Both latent and lytic EBV infection can be modelled in HIS mice (fig. 6). Depending on the viral strain, the majority of infected B cells either express all eight latent EBV proteins and only a minority initiates lytic replication (latency III predominates after B95-8 infection), or the frequency of lytically EBV replicating cells can be significantly increased (high lytic replication after M81 infection) [105]. The Hodgkin's and Burkitt's lymphoma-associated latencies I and II have been suggested to be present after HIS mouse infection with EBV [106–108], but they probably contribute to infection only at very low frequencies.

Apart from these virological parameters, some hallmarks of EBV specific immune control can also be recapitulated in HIS mice (fig. 6). The importance of NK cells in protecting from EBV-associated diseases, especially in the innate restriction of primary infection, was first demonstrated in HIS mice. Depletion of NK cells prior to infection

revealed a protective effect against lytic EBV replication [83, 95]. The importance of NK cells in recognising lytic replication during acute EBV infection was then also confirmed in longitudinal studies of paediatric patients with infectious mononucleosis [109], indicating the predictive nature of the humanised mouse model for EBV-associated diseases.

After the transient and protective NK cell proliferation, CD8<sup>+</sup> T cells massively expand during EBV infection of HIS mice [80, 89]. Both CD4<sup>+</sup> and CD8<sup>+</sup> T cells control viral loads, whereas CD8<sup>+</sup> T cells are more important in preventing EBV-associated lymphomas [80]. Among these expanding CD8<sup>+</sup> T cells, both latent and lytic EBV antigen specificities can be detected in HLA transgenic HIS mice [80, 90, 91]. Furthermore, a protective effect of EBV-specific T cell clones, which target lytic antigens against high viremia and extra-lymphoid lymphoma genesis, could be documented [91], suggesting the importance of these specificities for future vaccine development against EBV. Indeed, targeting of EBV antigens to DC populations is under investigation in HIS mice [85, 110]. However, these distinct antigen specificities are so far still difficult to modulate and the role of their effector functions cannot be assessed. The generation of recombinant viral vectors to manipulate the expression of certain genes in the developing haematopoietic lineage would constitute a valuable tool for assessing the importance of specific human immune functions during EBV-associated lymphoma genesis. For the investigation of adaptive cellular immune responses to EBV, T cell receptor transgenes would allow the development of T cell populations that could not only be tracked during the course of EBV challenge, but also further investigated for their protective potential during viral infection. Together with recombinant manipulation of the EBV

**Figure 6: HIS mice can be persistently infected with Epstein-Barr virus (EBV) and develop immune responses to the infection.** Mice that have been reconstituted by means of neonatal intrahepatic injection with human CD34<sup>+</sup> haematopoietic progenitor cells develop viraemia upon intraperitoneal EBV infection. Lytic replication deficient virus (BZLF1-) has a lower viral titre at 3 weeks after infection. NK cells expand to lytic EBV infection at 4 weeks after infection and CD8<sup>+</sup> T cells react to both lytic and latent infection with expansion by 5 weeks of infection.



genome [91, 95, 107, 111], the aforementioned strategies might lead to predictive preclinical models for relevant human diseases, which would provide insight that can be more readily translated into targeted clinical research.

### Human immunodeficiency virus infection in humanised mice

The human immunodeficiency virus (HIV) type-1 belongs to the lentivirus genus (family of retroviruses), comprising five serogroups that can infect a number of vertebrates. Clinical hallmarks of lentiviral infections are chronicity, intra-host evolution and cellular tropism [112]. Their basic genomic structure comprises the long-terminal repeat (LTR; transcription start), three genes encoding the structural proteins – gag, pol and env – and regulatory genes (Tat and Rev) [113]. Notably, accessory genes contribute to their virulence [114, 115].

HIV is a human-specific virus; the HIV pandemic counts currently 36.7 million of HIV-infected human (<http://www.who.int/hiv/en/>). HIV infects mainly CD4<sup>+</sup> T-cells and macrophages, leading to their progressive depletion [116, 117] and exhaustion [118, 119]. Moreover, HIV can remain latent by integrating its genome into the host chromosome, allowing viral rebound upon interruption of antiretroviral treatment (ART). Many *in vivo* aspects of HIV infection remain an enigma, e.g., HIV transmission [120], HIV latency [121, 122], tissue target cells [123–125], role of the HIV accessory genes [126], immune activation [127] and co-infection pathogenesis [128–130]. Of course, a key in any research efforts is the identification of novel therapeutic approaches [131–136].

The lack of a small HIV animal model has hampered HIV research since its discovery. The feline immunodeficiency virus (FIV), the simian immunodeficiency virus (SIV) and hybrid viruses between SIV and HIV (SHIV) have been used as a substitute for HIV in domestic cats or nonhuman primates, respectively. Findings with these models must further be validated with HIV itself. Notably, the “first” generation of hu mice, already used in the 1980s i.e., the SCID hu thy/liv or hu PBL SCID [137–142], lacked *de novo* multilineage haematopoiesis and/or suffered from graft-versus-host disease (GvHD), limiting their use in HIV research.

The “new” generation of hu mice (see introduction) are highly sensitive to HIV infection (figs 7 and 8) [97, 143–146] and recapitulate key features of HIV infection in human, e.g., dissemination [146], pathogenesis [147] and

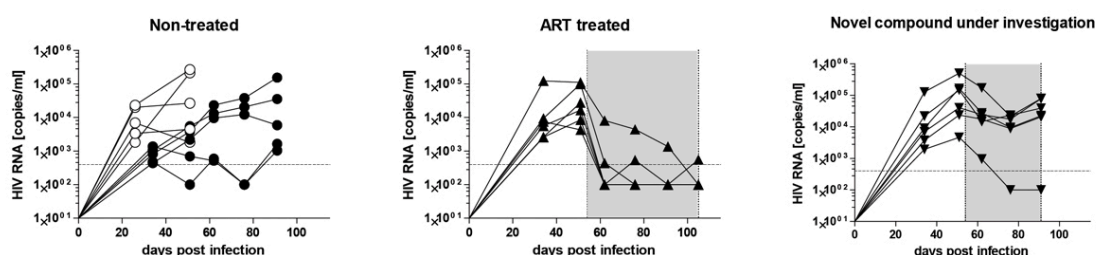
latency [148]. In the following, we will give a short update about the various humanized mouse models currently used in HIV research, including the pros and cons (figs 7 and 8). NSG mice intraperitoneally transplanted with adult human peripheral blood mononuclear cells comprise a simple and direct lymphoid-based model that allows HIV infection over 10 weeks [149]. This model is similar to the hu PBL SCID mouse model [4] and has similar limitations, i.e., lack of multilineage haematopoiesis and risk of GvHD. Thus, we would like to highlight only one study, which showed the value of this model to assess the presence of residual HIV reservoir in immunosuppressed patients, employed as a murine viral outgrowth assay [150]. Hu mice based on the transplantation of human CD34<sup>+</sup> HSPCs develop long-term *de novo* multilineage haematopoiesis and immunity [9] with low prevalence of GvHD. They are very valuable for long-term studies, studying HIV infection in deep tissues, HIV latency establishment [146, 148] and virus-specific CD8<sup>+</sup> T-cell development [92].

These mice have been very useful for dissecting the pathogenic role of the HIV accessory proteins and for studying HIV-associated immune activation. For example, by use of these mice, the destructive potential of a distinct motif of the Nef gene, the secretory modification region (SMR) by promoting CD4 apoptosis and a cytokine storm was convincingly demonstrated [151]. As related to HIV-associated immune activation, a recent study reported how exosomes containing transactivation response (TAR) RNA-stimulated macrophages and plasmacytoid dendritic cells (pDC), thereby promoting interferon-beta (IFN-β) and IL-6 secretion [152].

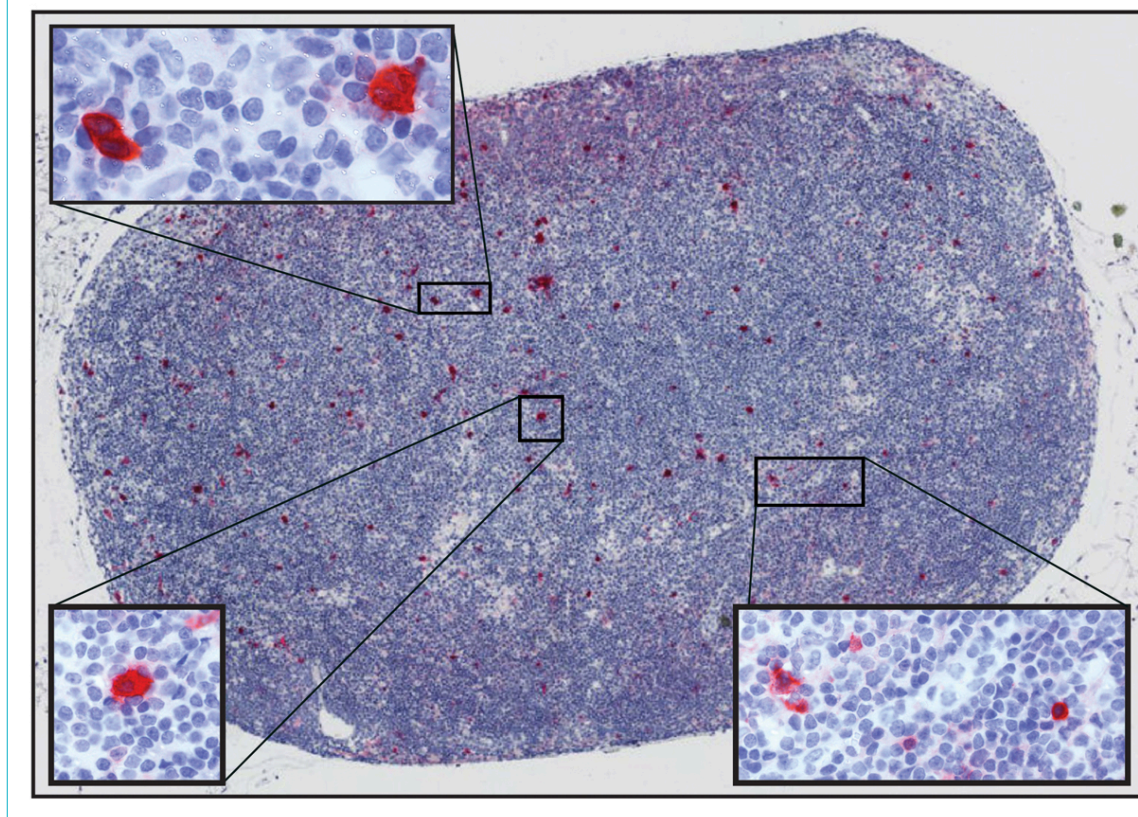
Notably, NSG mice have been successfully used to investigate novel treatment approaches (fig. 7), pre-exposure prophylaxis (PrEP) against vaginal transmission [153, 154], testing novel innovative compounds [155], optimised long-acting ART [156–158] or broadly neutralising antibodies (bNAb) [159]. Importantly, bNAb also reduced the latent reservoir size, either through FcγR engagement [160] or by additional treatment with latency reversing agents [161] – the latter was the first study that successfully targeted the latent reservoir for its eradication and thus a potential cure of HIV. Similarly, gene therapy-based approaches resulted in functional cure in NSG mice.

Bone marrow, liver, thymus mice (BLT) produced by concomitant transplantation of fetal liver/thymus tissues with CD34<sup>+</sup> cells into adult immunodeficient mice are a widely

**Figure 7: Humanised mice are highly susceptible to human immunodeficiency virus (HIV) and allow the examination investigational drugs.** Humanised mice were infected with the CCR5-tropic HIV strain, YU-2, and viral load was monitored over time with quantitative real-time PCR. The left panel shows untreated mice. The middle panel shows mice treated with antiretroviral therapy (tenofovir, lamivudine and raltegravir). The right panel shows mice treated with an investigational compound. The grey shaded area indicates the treatment period. The filled and empty circles in the untreated group represent mice from two independent cohorts.



**Figure 8: Human cells expressing p24 (dark red) in the lymph node of an HIV-infected NSG mouse.** The amplified image at the top left corner shows multi-nucleated giant cells, i.e., infected T cells that fuse with each other inducing syncytia. The lower left image shows a single HIV-infected macrophage. The lower right image depicts HIV-infected T cells among other HIV-infected cells.



used model. The thymic tissue resulted in an increase of T cell reconstitution, maturation and selection, enabling the generation of an MHC-restricted adaptive immune response from which HIV shows immune escape [162–164]. Notably, engraftment of human cells in the gastrointestinal tract and female reproductive tract is very prominent as compared with NSG mice, which permits study of HIV transmission and replication in mucosal tissues [165–169]. Viral replication was found preferentially in intestinal crypts, which released a pool of both immature and mature virions [170]. These findings were in contradiction to *in vitro* findings, where virion maturation occurred quickly, emphasising the value of *in vivo* investigations with the corresponding microenvironment [171].

Furthermore, BLT mice facilitated broad HIV transmission research; for example, ART reduced the amount of cell-free virus in the female reproductive tract and in the breast milk, overall decreasing the prevalence of HIV transmission [172, 173]. Another study showed that early ART initiation was able to preserve the immune system and functionally control productive HIV infection thanks to the development of a HIV-specific immune response [174, 175]. These results corroborated findings from the Visconti study in humans, where 14 patients treated during primary infection were able to constrain HIV infection for several years without treatment [174, 175].

Moreover, numerous prophylactic approaches have been validated using this model. For instance, lentiviral modification of HSPCs with a polymeric anti-HIV IgA construct or on adeno-associated virus (AAV) containing a bNAb (VRC07) hampered mucosal HIV transmission [176, 177].

Last, but not least, a study examining cross-species transmission events from chimpanzees to humans: Yuan et al. decoded critical viral envelop mutations required to allow HIV zoonosis [178]. Altogether, the BLT model is highly valuable, especially for mucosal tissues and adaptive immune response analysis.

More and more sophisticated humanised mice models are generated in order to recapitulate the human cell distribution and functionality in animals and which are examined for studying HIV. For example, MITRG and MISTRG mice demonstrated superior myeloid and NK cell development and function, in comparison to preceding models [17]. Deng et al., using MIS<sup>KI</sup>TRG mice demonstrated the potential of cytotoxic CD8<sup>+</sup> T cells (CTL) obtained from chronically infected patients, to constrain HIV released from reactivated latent cells [179].

To address more precise questions, highly specific humanised mouse models are used, such as, the T cell only and macrophage only mouse model (TOM and MOM, respectively) that permits development of only a specific human lineage. TOM can be readily infected with HIV and establishment of latency occurred in absence of other cell types [180]. Similarly, MOM confirmed the capacity of some strains of HIV to replicate and infect macrophages *in vivo* [181].

The studies mentioned above represent only a small fraction of insights gained in HIV infection/pathogenesis and cure using humanised mice that underscores their value for studying HIV. They likely represent the long awaited small animal model for the HIV research community. Since various models are available, and more will be available soon,



the HIV researcher will be faced with the challenge to identify the most suitable model to address their research question.

## Conclusions and outlook

Our understanding of human haematopoiesis and immunity has extensively evolved with the help of humanised mouse models. Currently, there are an increasing number of models for more aggressive neoplasms, such as AML and ALL, and for infectious diseases, such as EBV and HIV. Experimental strategies used in order to improve these and create new models include the expression of supportive human factors by genetic modification of mice, the co-transplantation of human mesenchymal stem cells, and the xenotransplantation of humanised ossicles. One particularly interesting approach is the use of the CRISPR/Cas9 technology for fast genetic modification of humanised host strains to express specific human factors. Despite on-going efforts, modelling less aggressive neoplasms, reproducing disease heterogeneity seen in patients, and probing certain immune cell pathologies is still a challenge that researchers face. Future models will need to support engraftment of the majority of patient samples for any given neoplasm or infection to allow researchers to draw substantial conclusions and predict accurate therapy responses in humans.

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